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THE ROLE OF BURSECTOMY IN THE CHICKEN ON ANTIBODY PRODUCTION

AGAINST NEWCASTLE DISEASE AND CHICKEN POX VIRUS

(Following is the translation of an article by U. Rundlein, Institute of Animal Hygiene and Fowl Diseases, Veterinary College, Hannover, published in the German language periodical Zent. Vet. 12B, pages 143-154 (65). Translation performed by Constance L. Lust.)

For some time it has been assumed that the thymus influences antibody formation. Numerous trials to test this hypothesis have so far all been negative. Harder (1948) Maclean (1957) and Harris (1956) found no difference in antibody formation against several different antigens between thymectomized and normal animals. In the thymus of immunized animals no elevated antibody content was found, which was the case for spleen and lymphnodes. (Bjorneboe 1947, Fagraeus 1948, Harris 1954, Trowell 1958). In thymocytes (Harris et al 1948) or in their tissue culture (Thorbecke and Keuning 1953) no antibodies were found.

In connection between thymic function and antibody syntheses appears probable if one considers the reports of various autoimmune illnesses of humans. If the thymus is lacking, or contains a tumor, there is a loss, or lack, of circulating gamma globulin (Good 1954, MacLean 1956, Ramos 1956, Martin 1957, Bader 1958, Gitlin 1959, Gafni 1960.)

Finally it was possible to show that early thymectomy in mice, rats, rabbits and guinea pigs is associated with a loss (total or partial) in formation of agglutinating, precipitating and hemagglutinating antibody. After sensitizing with antigens as S. typhimurium, influenza virus, T2 phage, sheep erythrocytes and BSA no antibodies were formed (Archer 1961, Michaelius 1961, Miller 1961, 1962 a,b, 1963, Archer 1962, Jankovic 1962, Papermaster 1962a, Miller 1964). The host reaction to a transplant (graft) was also slowed, or abolished (Miller 1961 1962 a, c, Arnason 1962, Arnason 1962, Dalmasso 1962 a, b, Martinson 1962). In the mammal a lymphopenia occurrs in blood as well as in the lymphatic organs (Paton 1904, Pappenheimer 1914, Klose 1914, Comsa 1957, Nakamoto 1957, Metcalf 1960, Miller 1961, Schooley 1961, Martinson 1962, Parrot 1962, Arnason 1962, Waksman 1962).

It was assumed that in the bird the function of the mammalian thymus is divided into the thymus and the Bursa fabricii which is also designated as "cloacal thymus" by Jolly (1920) (Burnet 1962, Papermaster 1962, Warner 1962). When the Bursa fabricii was removed surgically in early life (few days) an insufficient amount of antibody results (syndrome). This was reported by Chang, 1955,57,58,59, Glick 1956, Jankovic 1962, Mayer 1962, Perek 1962, Graetzer 1963, Kenenes 1963).

After injecting testosteron into incubating chicken eggs (between

the 5th and 12th day of incubation) the Bursa fabricii did not form (Meyer 1959, Perek 1960) and no antibody synthesis followed (Miller 1960, Warner 1961, Miller 1962, Papermaster 1962 b, Nemeness 1963). Ten percent of the animals showed atrophy of thymus along with the bursa. These animals lost the ability to reject skin grafts. Sixty percent had a normally developed thymus, but these animals made no antibodies against a group of antigens (Szenberg and Warner 1962).

Removal of thymus surgically had no clear influence on antibody synthesis. All thymectomized as well as all normal animals formed antibodies after stimulation with human gamma globulin (HGG) (Warner 1962). Other antigens eg. BSA gave the same result (Wolf 1963, Miller 1964, Graetzer 1963, Nemeness 1963).

Contrary to the results described above with bacterial antigens are BSA and HGG, if the bursa was removed in chickens, and then infected with NDV (B_1 strain) normal antibody formation was seen. Cho (1963) concluded that the bursa fabricii is not always involved in antibody formation, but that this depends on the type of antigen. It may be possible that in fowl the thymus is responsible for the synthesis of certain antibodies.

In extensive trials the effect of thymectomy of chickens on antibody formation was investigated during atypic NDV and chicken pox infection.

Methods and Materials

The whole experiments were performed with 124 HNL chicks. The eggs were obtained from NDV -and pox free colony and were hatched in the institute. For the NDV trial 60 chicks were used in groups of 10. For the pox trial 64 chicks were grouped per 16 animals. Half of the chicks were thymectomized at 3-6 days; the rest were controls. All chicks were kept in incubators and fed pellets of food.

Operation: Anesthetic after Freytag (1963) with nembutal diluted 1:6 with saline; dose of 0,005 ml per gram body weight IM. Median cut dorsal from atlas to chest, the thymus knots were removed with a bent forceps. Souture with 4 stitches (not removed).

Newcastle Disease Virus: The Hitchner B_1 strain of ND vater vaccine (Behring Co.) was used. The dose for 100 animals was disolved in 100 ml H_2O and every animal received 1 ml by pipette into the gullet. The chicks were challenged with highly virulent field virus. Every chick was injected with 1 ml of 1:50 dilution (10^6 egg LD IM).

Chicken Pox: Chicks were vaccinated into wings with type H. Challenge with strain C 360, 1 ml 1:dp dilution IV. Titer was 10^5 egg LD corresponding 320 cell agglutination units (Kanguide and Hanson 1961).

Serology: Blood was obtained by left-side heart puncture 4 weeks post vaccination and two weeks post infection. Serum was stored at -20°C and used as: 1) hemagglutination (HI) inhibition diluted 1:5 - 1:20,840,

2) [illegible] precipitation [illegible] and 1950), 3) Neutralization test [illegible] was chosen because it conserves [illegible] and [illegible] the antibody concentration [illegible] and Horsfall 1950).

A single vaccination with Hitchner B1 was done at 3 weeks of age, infection 3 months later. Mild illness without great danger of illness occurred.

Statistics: The [illegible] titers of control and thymectomized groups were compared by the t-test. The median value and standard deviation and significance were calculated according to Mudra (1950).

Since [illegible] and HI titration was done the statistical could not be computed on the original data. For a dilution of 1:5 SNT or HI a value of 1; 1:20 = 2,) 20 = 3. ADP + positive value = 1 negative = 0; they were calculated with the "chi" square test after Yates (1940).

Results

Natural Disease: Without vaccine no differences in clinical course after infection with field virus was seen between the normals and thymectomized. Illness was severest at day 3 and 6 in both groups, (loss of appetite, dyspnea, diarrhea). In the following 3 days all but one chick in each group died.

After infection after vaccination, likewise no difference was seen. Incubation and course of illness correspond to the unvaccinated group. Mortality was clearly lowered. Forty-five per cent of 20 Hitchner B1 vaccinated animals (5 thymectomized and 4 controls) died in the first week after infection.

During the pathology study all dead animals showed typical signs. In most chicks hyperemic and bleeding of the organs was observed.

A table of the serologic results is presented in table 3 (statistical analysis in table 4).

The following conclusions are made:

In controls not vaccinated, not infected, no positive ADP or HI or SNT tests.

Of those vaccinated with Hitchner B1 not all precipitated sera, except one thymectomized chick 4 weeks post vaccination.

HI titers were positive in 4 thymectomized animales (1:40, 1:10, 1:10, 1:10) and in 3 normals. Neutralizing antibodies were demonstrated for 1 (1:20) thymectomized and 1 normal (1:5). The elevated antibody content in the normal animals 4 weeks post vaccination is not statistically

significant. Fifteen weeks after vaccination all sera were negative in the precipitation test.

HI titers were positive in 4 of 5 sera in both groups. Generally the small differences seen were not significant.

The difference in formation of neutralizing antibodies was clearer. In the normal group all 5 animals were positive (1:10, 1:5, 1:5, 1:5, 1:5); in the thymectomized only 2 of 5 (1:5, 1:5). This difference is statistically weakly significant. Two weeks after NDV infection with virus, after previous vaccination with Hitchner B1, no significant differences were seen.

Five thymectomized and 4 of 5 normals gave positive sera in HIP. Sera of all chickens -also the thymectomized- had high NT and HI titers (between 1:640 and 1:5120).

It was assumed that full immunity against NDV is probable so long the HI titer is at a level of 1:5. (Bengelsdorff 1963). However, 15 weeks after vaccination of chicken which had HI titers of 1:10, experimental infections resulted in a high mortality and illness in almost all. To be sure these animals were infected parenterally with high doses of virus. In the infected group which had not been vaccinated (high mortality) no serologic studies were done.

Pox

No differences were seen in the formation of a local reaction between normals and thymectomized animals. In all cases the reactions could be read easily on day 7 post vaccination. The clinical picture also looked the same. The first pox appeared on the comb on day 5 in the vaccinated group, on day 16 the normal signs could be seen in all 16 animals. One thymectomized chicken died on day 13. None of the vaccinated chickens became ill after infection with pox.

A review of the serologic results are presented in table 5. The following can be concluded.

The precipitation tests were negative in controls (neither vaccinated nor infected); also in the vaccinated animals this was as expected.

Two weeks after the IV infection with field virus (after vaccination) all sera of thymectomized animals gave negative precipitive test. In controls 3 of 8 chicks -sera precipitated. But, this difference is not significant. Smaller still was the difference in the non-vaccinated chickens; 2 weeks post infection precipitive tests were positive in sera of 4 of 8 thymectomized and 5 of 8 normal chickens.

It must be definite that neither the bursa fabricii nor the thymus plays a dominant role in humoral antibody formation against atypic chicken NDV. The thymus also does not influence immunity in chicken pox. It should be mentioned that even with much care during thymectomy of chickens

small pieces of thymus may remain in the animal (Warner 1960, Aspinall 1963). These small bits may cause an influence even though it be reduced. Warner et al (1960) and Aspinall (1962) used this concept to explain their data concerning protracted, incomplete rejection of skin grafts in chickens.

Because of this the differences in report must be considered, even though they may not be conformatory. Therefore the differences in antibody formation 15 weeks post vaccination with Hitchner B1 virus, or infection with pox, should be considered even though they are not statistically significant.

The possibility does exist that this small difference in antibody titer against Hitchner B1, is not due to a protracted formation, but due to an increased rebuild of the antibody level after thymectomy.

Since the local vaccine-reaction, the level of protection, the clinical picture was the same, or only slightly different (not statistically significant), one cannot conclude unequivocally that the thymus has an important function in antibody formation in chicks against NDV or chicken pox.

Summary

After inoculation and infection with the virus of ND and FP no significant differences were detected between thymectomized and not thymectomized chickens with respect of the reaction to vaccination, formation of humoral antibodies, degree of immunity, disease pattern and mortality.

It is worth pointing out that in the thymectomized chickens infected with FP the number of positive precipitation tests, particularly in the vaccinated birds, was somewhat less than in the control birds and that 15 weeks after vaccination with Hitchner B1 ND virus the neutralization (~~0.05; P< 0.01~~) and HI titers were also lower in the thymectomized birds.

Figure 1.

Chicks before thymectomy. Both thymus surends visible

Table 1

Experiment with Newcastle Disease Virus - Number and age infected

		(1) Tiere pro Gruppe	(2) thymektomiert im Alter von	(3) vacciniert im Alter von	(4) infiziert im Alter von	(5) Anzahl der Blutentnahmen	(6) Blutentnahmen im Alter von
Kontrolle	nicht thymektomiert	5				5	2 W [illegible] 18 W
	thymektomiert	5	3—6 T			5	2 W 7 W 16 W
Hitchner B 1	nicht thymektomiert	5		3 W		5	7 W 18 W
	thymektomiert	5	3—6 T	3 W		5	7 W 18 W
Hitchner B 1 Feldvirus	nicht thymektomiert	10		3 W	16 W	5	18 W
	thymektomiert	10	3—6 T	3 W	16 W	5	18 W
Feldvirus	nicht thymektomiert	10			16 W	5	
	thymektomiert	10	3—6 T		16 W	5	

T = Tage W = Wochen

(1)-animals per group (2)-thymectomized at age (3)-vaccinated age
(4)-infected age (5)-blood taken (6)-age

Table 2

Experiment with pox Virus

		(1) Tiere pro Gruppe	(2) thymektomiert im Alter von	(3) vacciniert im Alter von	(4) infiziert im Alter von	(5) Anzahl der Blutentnahmen	(6) Blutentnahmen im Alter von
Kontrolle	nicht thymektomiert	8				8	2 W 5 W 14 W
	thymektomiert	8	3—6 T			8	2 W 5 W 14 W
Pocken-Vaccine	nicht thymektomiert	8		3 W		8	5 W 14 W
	thymektomiert	8	3—6 T	3 W		8	5 W 14 W
Pocken-Vaccine Feldvirus	nicht thymektomiert	8		3 W	12 W	8	14 W
	thymektomiert	8	3—6 T	3 W	12 W	3	14 W
Feldvirus	nicht thymektomiert	8			12 W	8	14 W
	thymektomiert	8	3—6 T		12 W	6	14 W

T = Tage W = Wochen

(1)-animals per group (2)-thymectomized at age (3)-vaccinated age
(4)-infected age (5)-blood taken (6)-age

Table 3

Antibody [illegible] and mortality in thymectomized and [illegible] animals ([illegible] vaccination with Hitchner B_1 and infection with virulent ND [illegible] virus. N-Neutralisation test, Agar-gel precipitation [illegible] test.

			1	2	3	4	5	Mortalität in der 1. Woche p.i.
Kontrolle	nicht thymektomiert	N-Test	—	—	—	—	—	
		NAH	—	—	—	—	—	
		AGP	—	—	—	—	—	
	thymektomiert	N-Test	—	—	—	—	—	
		NAH	—	—	—	—	—	
		AGP	—	—	—	—	—	
Hitchner B_1 4 Wochen p.i.	nicht thymektomiert	N-Test	1: 5	—	—	—	—	
		NAH	1: 10	1: 10	1: 5	—	—	
		AGP	—	—	—	—	—	
	thymektomiert	N-Test	1: 20	—	—	—	—	
		NAH	1: 40	1: 10	1: 10	1: 10	—	
		AGP	+	—	—	—	—	
Hitchner B_1 15 Wochen p.i.	nicht thymektomiert	N-Test	1: 10	1: 5	1: 5	1: 5	1: 5	
		NAH	1: 10	1: 10	1: 10	1: 10	—	
		AGP	—	—	—	—	—	
	thymektomiert	N-Test	1: 5	1: 5	—	—	—	
		NAH	1: 5	1: 5	1: 5	1: 5	—	
		AGP	—	—	—	—	—	
Hitchner B_1 Feldvirus	nicht thymektomiert	N-Test	1:5120	1:2560	1:2560	1:2560	1:1280	
		NAH	1:2560	1:1280	1:1280	1:1280	1: 640	50%
		AGP	+	+	+	+	—	
	thymektomiert	N-Test	1:5120	1:2560	1:2560	1:1280	1: 640	
		NAH	1:2560	1:1280	1:1280	1:1280	1:1280	40%
		AGP	+	+	+	+	+	
Feldvirus	nicht thymektomiert	N-Test						
		NAH						90%
		AGP						
	thymektomiert	N-Test						
		NAH						90%
		AGP						

Table 4

Statistical evaluation of the results in table 3

				1	2	3	4	5	6 Mittelwerte	7 Standard-Abweichung	8 Test χ^2-Werte	9 Statistische Signifikanz*)
Kontrolle		nicht thymektomiert	N-Test	—	—	—	—	—	0	0	0	—
			HAH	—	—	—	—	—	0	0	0	—
			AGP	—	—	—	—	—			$\chi^2 = 0$	—
		thymektomiert	N-Test	—	—	—	—	—	0	0		—
			HAH	—	—	—	—	—	0	0		—
			AGP	—	—	—	—	—				—
Hitchner B 1 4 Wochen p.i.		nicht thymektomiert	N-Test	1	—	—	—	—	0,20	± 0,45	0,63	—
			HAH	2	2	1	—	—	1,00	± 1,00	1,29	—
			AGP	—	—	—	—	—			$\chi^2 = 0$	—
		thymektomiert	N-Test	3	—	—	—	—	0,60	± 1,34		—
			HAH	4	2	2	2	—	2,00	± 1,41		—
			AGP	—	—	—	—	—				—
Hitchner B 1 15 Wochen p.i.		nicht thymektomiert	N-Test	2	1	1	1	1	1,20	± 0,45	2,52	+
			HAH	2	2	2	2	—	1,60	± 0,89	1,78	—
			AGP	—	—	—	—	—			$\chi^2 = 0$	—
		thymektomiert	N-Test	1	1	—	—	—	0,40	± 0,55		—
			HAH	1	1	1	1	—	0,80	± 0,45		—
			AGP	—	—	—	—	—				—
Hitchner B 1 ND-Feldvirus		nicht thymektomiert	N-Test	11	10	10	10	9	10,00	± 0,71	4,67	—
			HAH	10	9	9	9	8	9,00	± 0,71	0,56	—
			AGP	1	1	1	1	—			$\chi^2 = 0$	—
		thymektomiert	N-Test	11	10	10	9	8	9,60	± 1,28		—
			HAH	10	9	9	9	8	9,20	± 0,89		—
			AGP	1	1	1	1	1				—
ND-Feldvirus		nicht thymektomiert	N-Test									—
			HAH									—
			AGP									—
		thymektomiert	N-Test									—
			HAH									—
			AGP									—

6-mean 7-standard deviation 8-χ^2 9-significance

N-neutralization, HAH-HI AGP-agar gel precipitin test

		1	2	3	4	5	6	7	8	positive AGP in %	χ^2	[illegible]
[illegible]	nicht thymektomiert	—	—	—	—	—	—	—	—	0		—
	thymektomiert	—	—	—	—	—	—	—	—	0		
[illegible]	nicht thymektomiert	—	—	—	—	—	—	—	—	0		—
	thymektomiert	—	—	—	—	—	—	—	—	0		
[illegible]	nicht thymektomiert	+	+	+	—	—	—	—	—	37,5	1,64	—
	thymektomiert	—	—	—	—	—	—	—	—	0		
[illegible]	nicht thymektomiert	+	+	+	+	+	—	—	—	62,5	0,[illegible]	—
	thymektomiert	+	+	+	+	—	—	—	—	50,0		

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